Supporting Information

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SI Text

Determining Aneuploidy and Other Measurements Performed on Samples from Evolved Populations. Throughout this study, in cases where we analyzed an evolved population, we always measured the population and not a single clone. This is so that the measurements reflect the mean value for all of the population and not of a potential subpopulation from which a single clone could have been taken for measurement. Because we measure a population of cells, in the cases of determining aneuploidy we had to assess the part of the population that contains the reported aneuploidy by comparing it to a microarray of a monoclonal strain with the same aneuploidy. In all cases where we report aneuploidy, the estimated part of the population that is reported to either gain or eliminate a chromosome is at least 95%, as judged by the comparison with the reference strain. Nonetheless, in some cases, the population was more heterogenic and indeed we note that the trait has not yet been entirely fixated (see for example Fig. 5*C*, Refined 3 and 4).

Heat Tolerance Measurements: Liquid Growth vs. Heat-Shock Survival. In this study, we use two assays to compare heat tolerance of different strains: liquid growth and heat-shock survival. The liquid growth curve comparisons in the various conditions are the most suitable way to compare evolved strains, because evolution itself is performed via liquid growth under defined conditioned. Thus, our method is based on the notion that the test should be in the same setup in which the evolutionary process took place. Also, when comparing two strains that did not go through evolution, it is legitimate to compare them by growth rate analysis (Fig. 3*D*). However, when we compare a strain that evolved via liquid growth transfer to another strain that did not go through such an evolutionary process (e.g., when comparing *evo39* and *WTtrisomeIII* on heat stress), we must not use the growth rate analysis because during the evolution, strains adapt to the liquid growth itself in addition to the adaptation to the high temperature. Thus, in such cases the more reliable way to compare heat tolerance is to measure heat-shock survival ratio (as done in Fig. 3*A*). In addition, when appropriate, each heat survival analysis in Fig. 3*A* is also backed up by growth rate comparison in Fig. 3*D* and in Fig. S2.

Selection of Genes from Chromosome III That Retain High Expression Level After the Elimination of Chromosome III Trisomy Under Heat. We defined the genes from chromosome III that retained high expression level, despite the trisomy elimination, based on the four trisomic populations that eliminated chromosome III trisomy during 1,000 generations under heat (*Refined 1–4*). Only genes that maintained average $\log_2$ expression change (compared with WT) above 0.46 in at least three of the four repetitions were selected. The 0.46 criterion is based on the average expression increase in chromosome III genes observed in the trisomic *evo39* (compared with its diploid ancestor), which was 0.4562 ($\log_2$). This definition led to 23 genes from chromosome III that retained high expression, of which 17 were available in the Molecular Barcoded Yeast plasmid library (1). These genes were examined in Fig. 3. For these genes, gene ontology analysis did not show any significant term compared with all other genes on chromosome III.

Heat Tolerance Functional Analysis of Genes from Chromosome III That Retain High Expression Level After Elimination of Chromosome III Trisomy Under Heat. We have focused on the genes with the most substantial heat tolerance contribution when inserted singly into a wild-type cell (Fig. 4*C*). For each of these genes, we looked for further evidence to support their substantial heat tolerance contribution. Reassuringly, we indeed find that YCR065W (*HCM1*), which had the highest heat tolerance contribution (23.5%), was reported in a study to confer increased heat tolerance when overexpressed in yeast (2). For YCR045C (*RRT12*), which had 19.3% heat tolerance contribution, we preformed a heat-shock expression profile (mRNA abundance) and found an induction of more than 27-fold upon 45 min of heat shock (42 °C). We performed the same method for YCR071C (*IMG2*), which had 18.4% heat tolerance contribution and observed induction of 1.5-fold upon 90 min of heat shock. For these last two genes, we also found a study that reports heat sensitivity phenotype upon deletion (3). Two of the five most-heat-contributing genes that we found on chromosome III are still uncharacterized ORFs in yeast, yet they also show high responsiveness to heat shock. Interestingly, YCR016W (19.9% heat tolerance contribution) shows a fast, strong repression upon 15 min of heat shock, which is followed by a growing induction as the heat persists, and YCR102C (18.8% heat tolerance contribution) shows a 3-fold induction upon 90 min of heat shock.

1. Ho CH, et al. (2009) A molecular barcoded yeast ORF library enables mode-of-action analysis of bioactive compounds. *Nat Biotechnol* 27(4):369–377.
2. Rodriguez-Colman MJ, et al. (2010) The forkhead transcription factor Hcm1 promotes mitochondrial biogenesis and stress resistance in yeast. *J Biol Chem* 285(47): 37092–37101.
3. Sinha H, et al. (2008) Sequential elimination of major-effect contributors identifies additional quantitative trait loci conditioning high-temperature growth in yeast. *Genetics* 180(3):1661–1670.

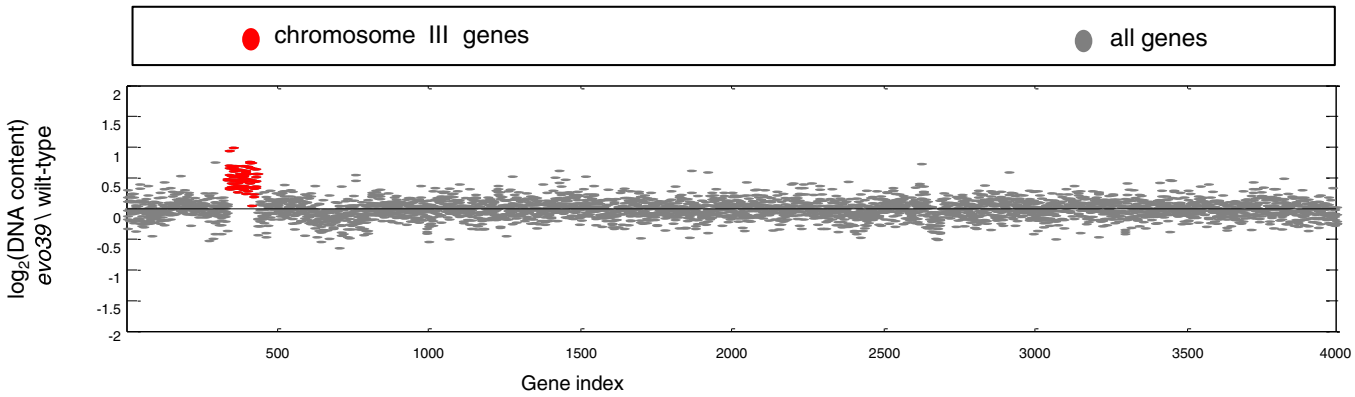

Fig. S1. Verification of chromosome III trisomy in *evo39*. Shown here is the elevated DNA content level of the segment that spans chromosome III. Dots represent $\log_2$ intensity ratios of DNA copy number, measured by DNA hybridization microarrays, of *evo39* over a diploid wild type, aligned according to chromosomal order where red dots represent genes from chromosome III and gray from all other chromosomes.

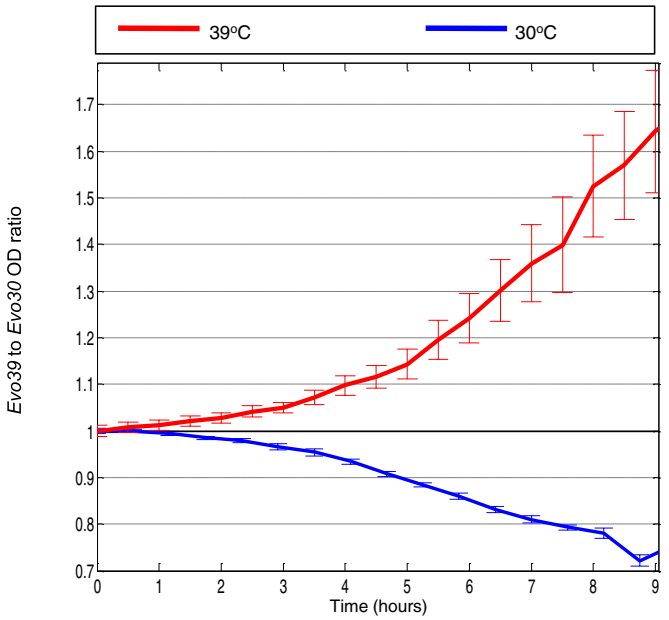

Fig. S2. The benefit and cost of chromosome III trisomy. *Evo39*, a strain that evolved 450 generations under heat and gained an extra copy of chromosome III, is growing better under heat than *evo30*, a strain that evolved at permissive temperature for the same number of generations and remained euploid. The results are reversed when measuring growth at 30 °C; when heat is not applied, the extra copy of chromosome III decreases the growth of *evo39* compared with the euploid *evo30*. Values represent OD ratios of *evo39* over *evo30* measured during continuous growth at 39 °C (red) and at 30 °C (blue). Data are presented as mean and SEM.

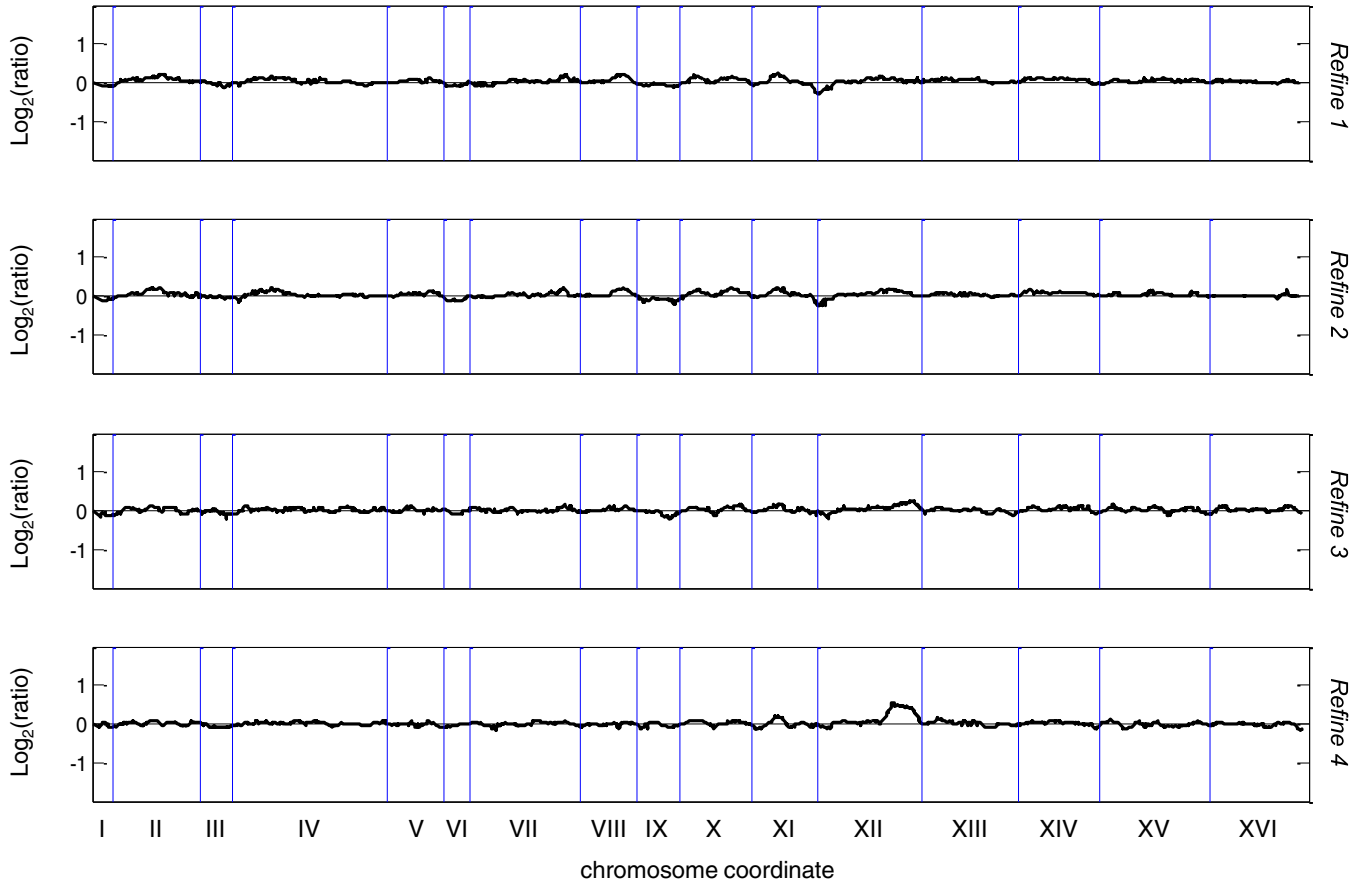

Fig. S3. Chromosome III trisomic strains, further evolved under heat, eliminated the trisomy. Four independent repetitions, descendants of *evo39* (*Refined 1* and *2*) and *WTtrisomeIII* (*Refined 3* and *4*), were further evolved for 1,000 generations under heat (39 °C) and minimal medium. All lines represent $\log_2$ intensity ratios of mRNA abundance, calculated by a sliding window of heat-evolved strain over a diploid wild type, aligned according to chromosomal order where blue vertical lines differentiate between chromosomes. Whereas chromosome III trisomy was eliminated in all four repetitions, in *Refined 4* it appears that a subpopulation also duplicated a segment in chromosome XII. Interestingly, *Refined 4* shows the lowest improvement under heat and the lowest cost reduction compared with the other *Refined 1–3* (Fig. 4*A*).

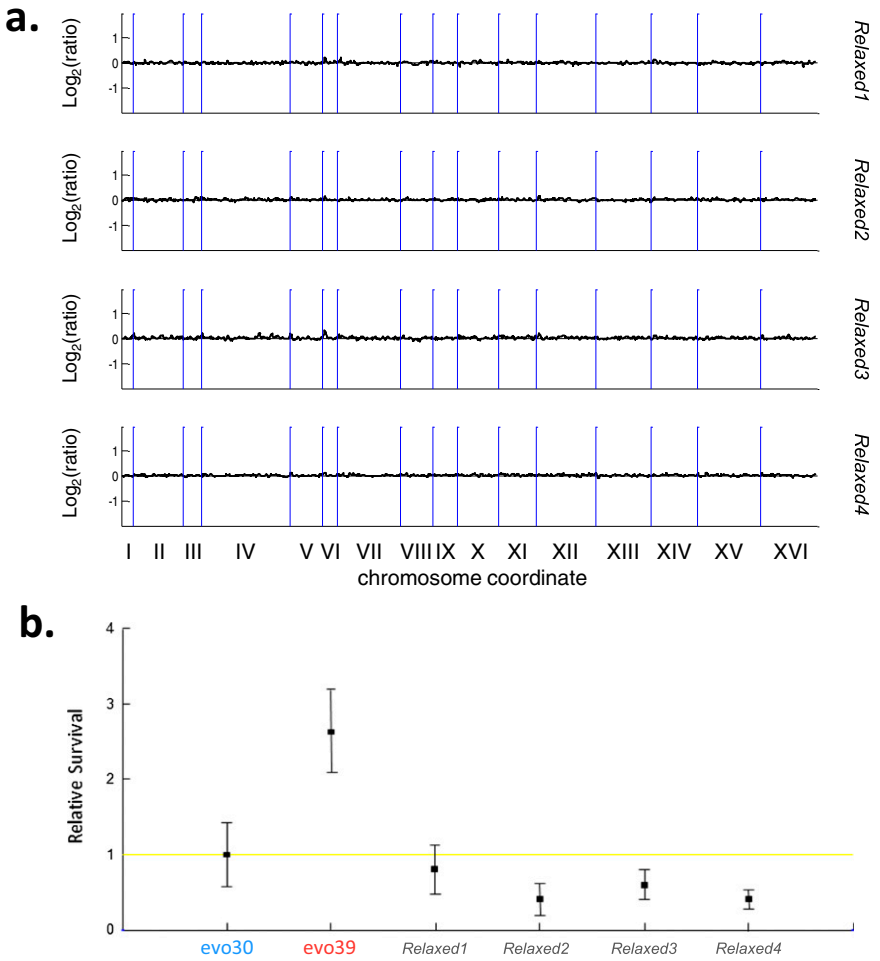

Fig. S4. Chromosome III trisomic *evo39* strain, further evolved at permissive temperature eliminated the trisomy and lost its increased heat tolerance. Four independent repetitions, descendants of *evo39*, were further evolved for 600 generations under 30 °C and rich medium (defined as *Relaxed 1–4*). (*A*) Chromosome III trisomy was eliminated in all four repetitions. Black lines represent $\log_2$ intensity ratios of mRNA abundance calculated by a sliding window of permissive temperature-evolved strain over a diploid wild type, aligned according to chromosomal order where blue vertical lines differentiate between chromosomes. (*B*) Heat-shock tolerance rates decreased with the elimination of chromosome III trisomy. Shown is the heat-shock survival fold change of chromosome III trisomic *evo39* and its four descendants *Relaxed 1–4*. Yellow line represents the heat-shock survival of *evo30* that was evolved in parallel to *evo39* but at 30 °C and it remained euploid. Data are presented as mean and SEM.

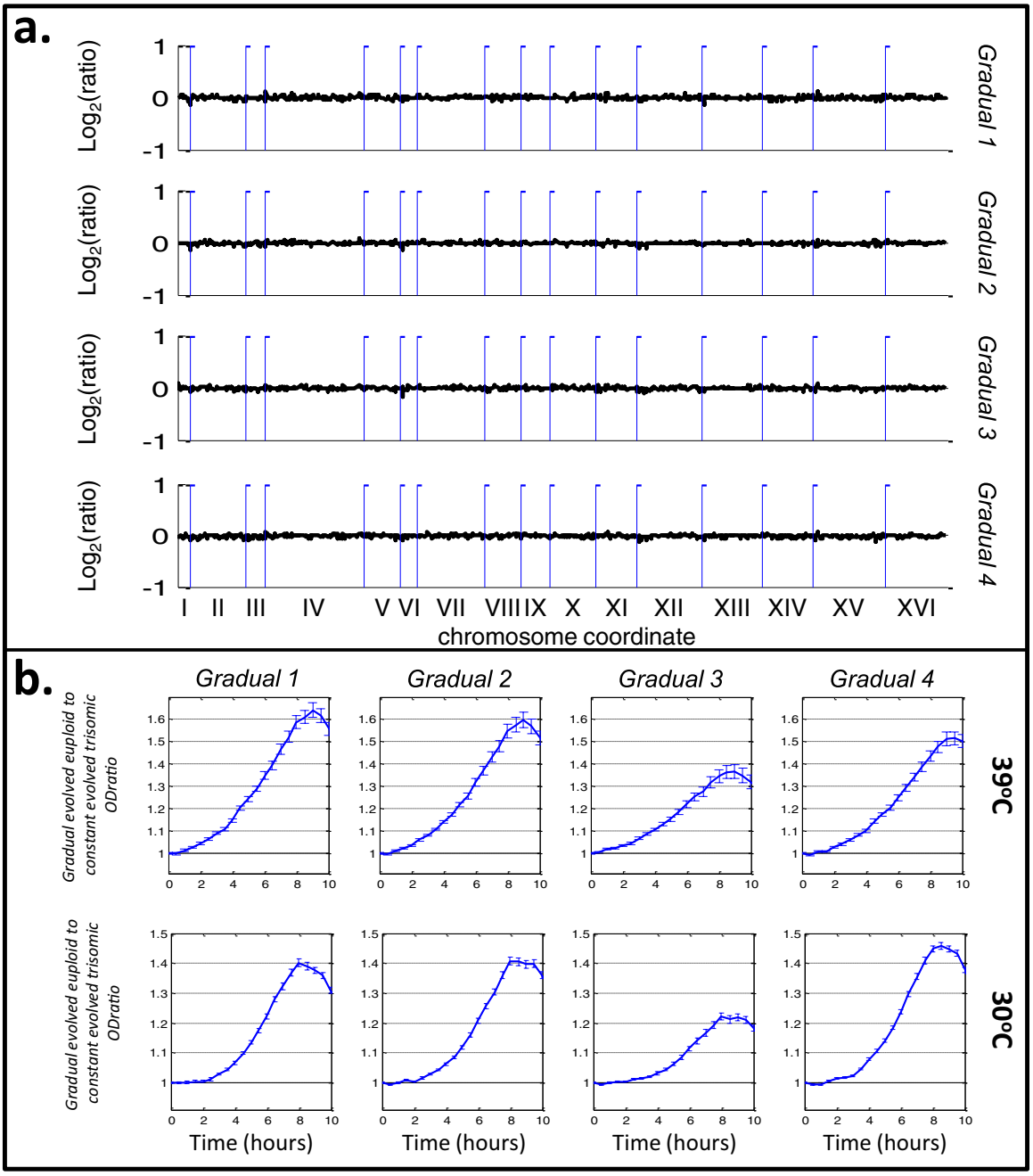

Fig. S5. Cells evolved under gradually applied heat do not adopt aneuploidic solutions. The laboratory evolution experiment in which four independent repetitions were evolved under 39 °C and duplicated chromosome III (*H1–4*) was repeated under the same conditions but heat was applied in a gradual manner, in four independent repetitions (*Gradual 1–4*). For these strains evolution started at 30 °C and every 50 generations the temperature was raised by 1 °C. Total number of generations was 450, identical to the number of generations in the case of *H1–4*. (*A*) All *Gradual 1–4* remained euploid, i.e., chromosome III trisomy was not detected in any of the four repetitions. Black lines represent $\log_2$ intensity ratios of mRNA abundance, calculated by a sliding window, of gradual heat-evolved strain over a diploid wild type, aligned according to chromosomal order where blue vertical lines differentiate between chromosomes. (*B*) Comparing the growth of the euploid *Gradual 1–4* to the growth of the trisomic *H1–4* shows better growth of the gradual evolving strains both at 39 °C and at 30 °C. Each subgraph shows the OD ratios of one of the gradual strains over its nongradual counterpart, measured during continuous growth at 39 °C (*Upper*) and at 30 °C (*Lower*). Data are presented as mean and SEM.

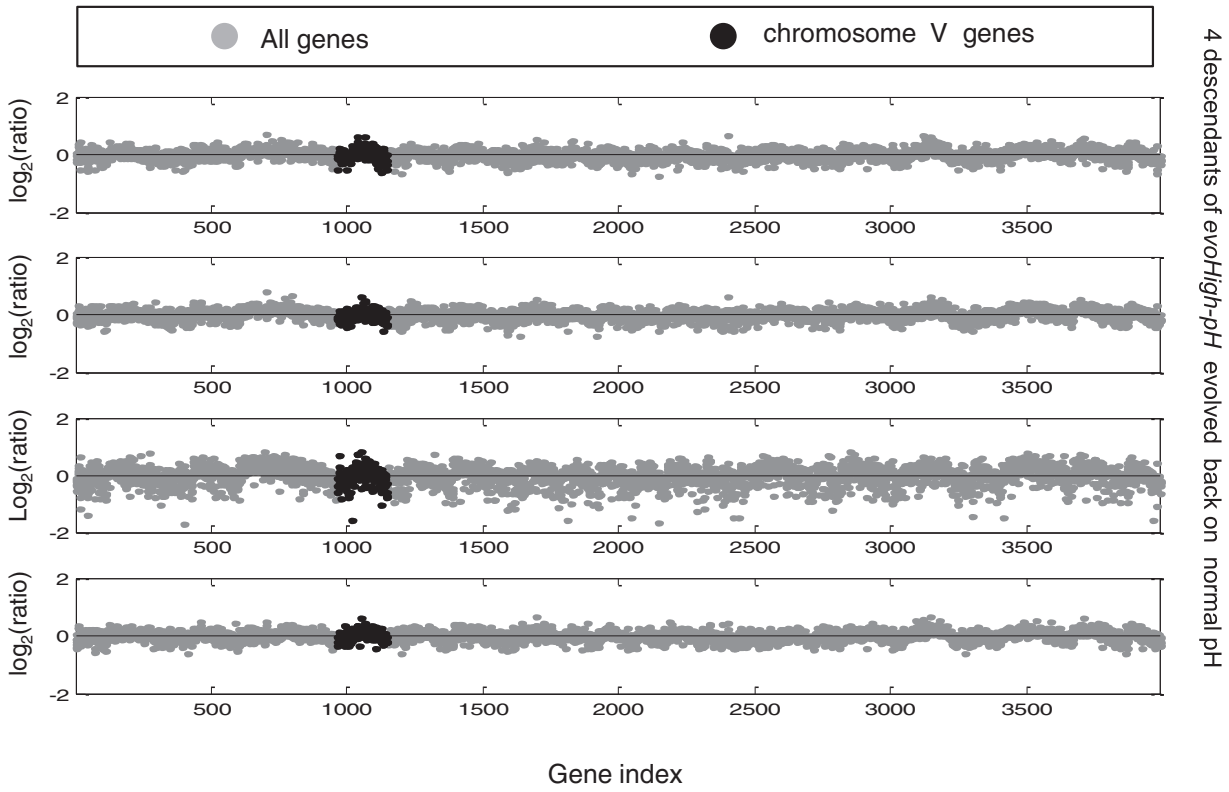

Fig. S6. Chromosome V disomic *evoHigh-pH* strain eliminated the disomy after further evolution at normal pH (6.7). Four independent repetitions, descendants of *evoHigh-pH*, evolved for 280 generations under pH 6.7 were analyzed and chromosome V disomy was eliminated in all repetitions. Dots represent $\log_2$ intensity ratios of DNA copy number over a haploid wild type, aligned according to chromosomal order where black dots represent genes from chromosome V and gray from all other chromosomes.

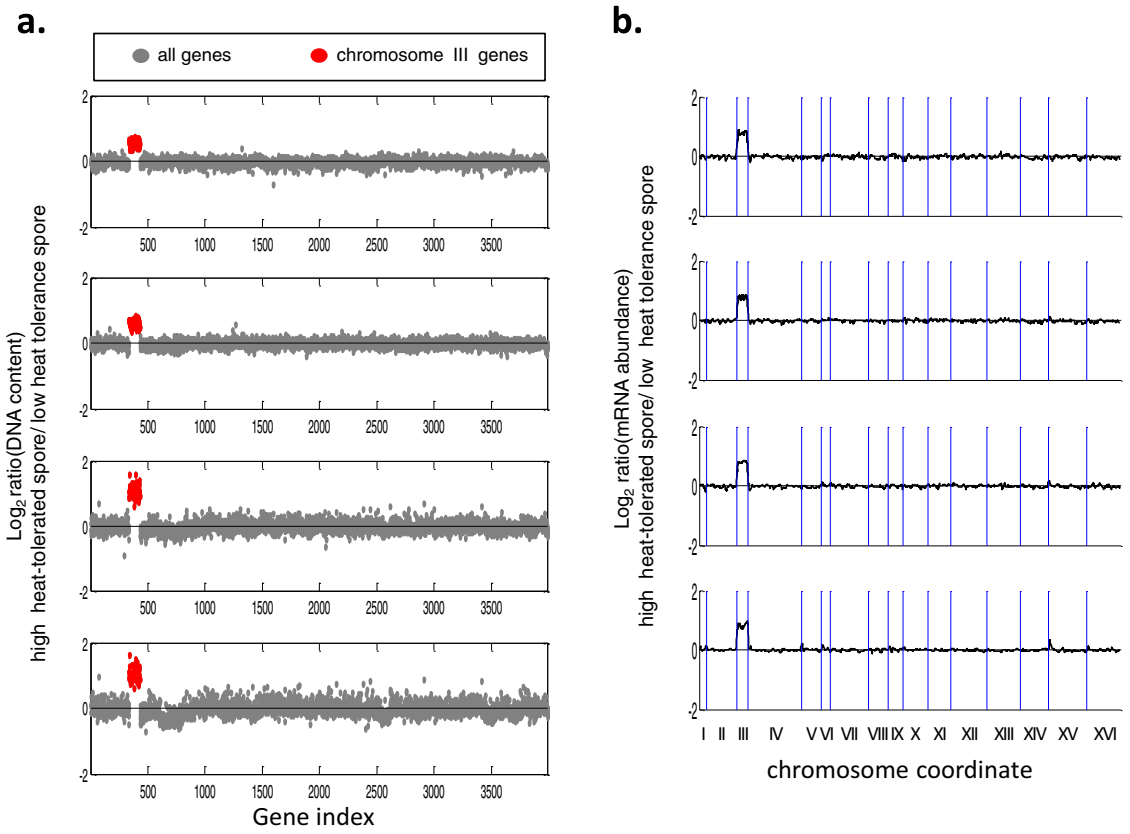

Fig. S7. Determination of extra chromosome III segregation in *evo39* and *WTtrisomeIII* spores. (*A*) *WTtrisomeIII* was subjected to tetrad analysis and the heat tolerance of eight spores from two tetrads was measured. Spores were divided into pairs according to their heat tolerance; each pair consists of one spore that showed increased heat tolerance and one spore with a heat tolerance level similar to that of a haploid wild type. Dots represent $\log_2$ intensity ratios of DNA copy number of increased heat tolerance spore over wild-type–like heat tolerance spore, aligned according to chromosomal order, where red dots represent genes from chromosome III and gray from all other chromosomes. (*B*) *Evo39* was subjected to tetrad analysis and the heat tolerance of eight spores from two tetrads was measured. Spores were divided into pairs according to their heat tolerance; each pair consists of one spore that showed increased heat tolerance and one spore with a heat tolerance level similar to that of the wild type. Dots represent $\log_2$ intensity ratios of mRNA abundance calculated by a sliding window of increased heat tolerance spore over wild-type–like heat tolerance spore, aligned according to chromosomal order where blue vertical lines differentiate between chromosomes.

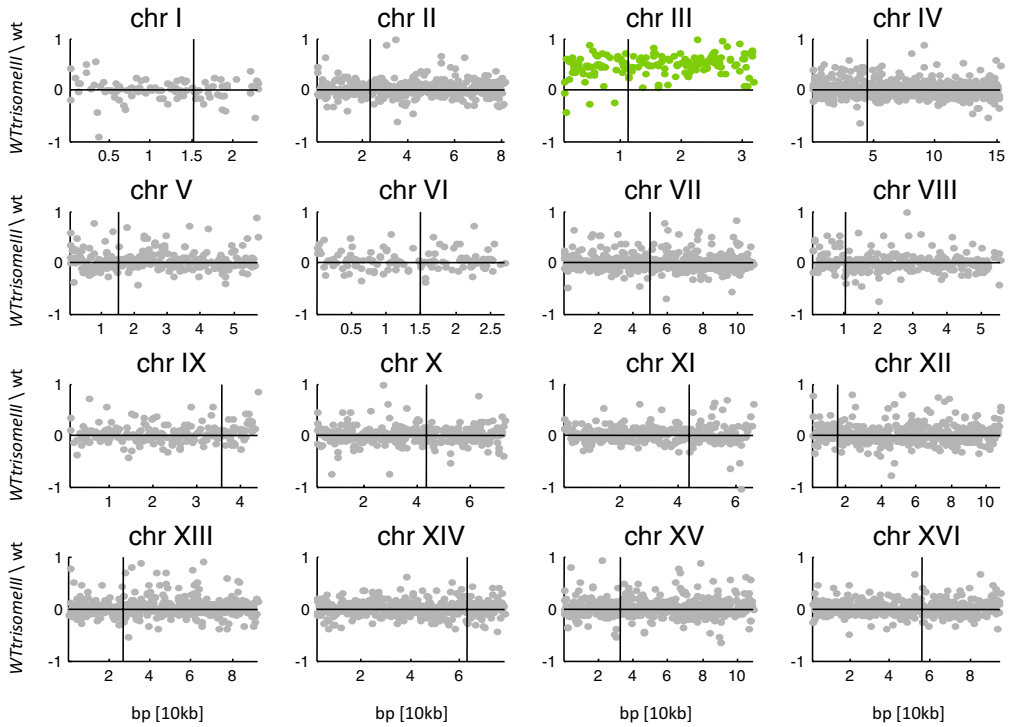

Fig. S8. Verification of *WTtrisomeIII* strain to be trisomic for chromosome III. Dots represent $\log_2$ intensity ratios of *WTtrisomeIII* to a diploid WT from expression microarray, for each of the 16 chromosomes and aligned according to chromosomal order. Green dots represent genes from chromosome III and gray from all other chromosomes. The vertical line within each chromosome represents the centromere position. Whereas in all chromosomes the average ration is around zero (indication for the identical chromosome copy number), for chromosome III there is ~50% higher expression, an indication of three copies of chromosome III compared with two copies in the WT.

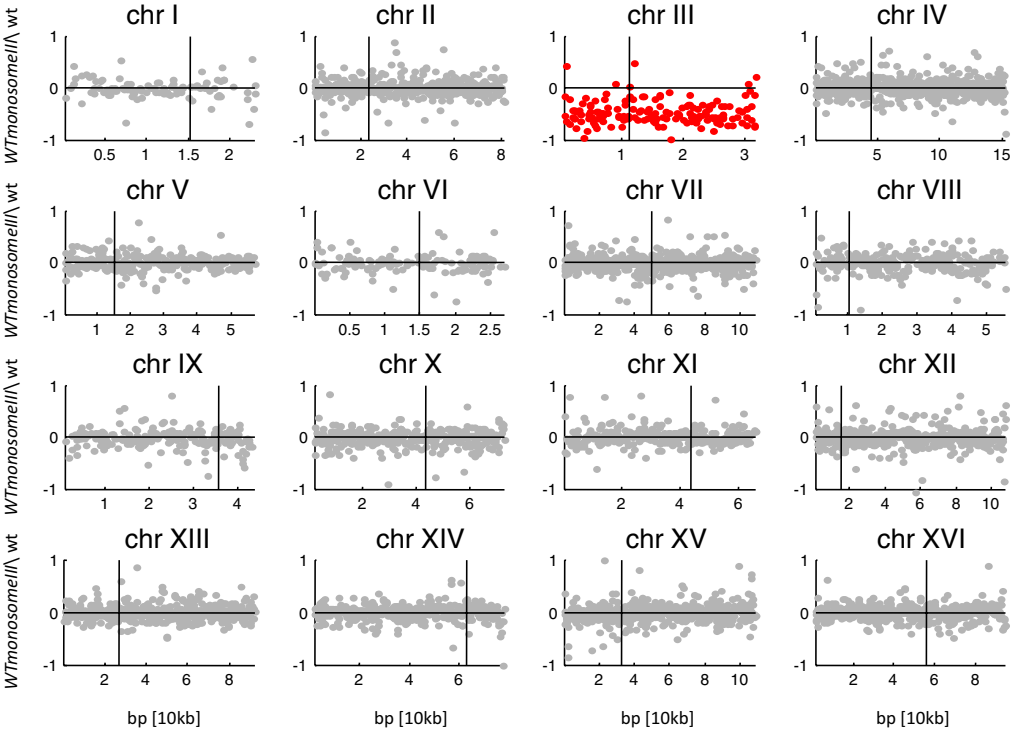

Fig. S9. Verification of *WTmonosomeIII* strain to be monosomic for chromosome III. Dots represent $\log_2$ intensity ratios of *WTmonosomeIII* to a diploid WT from expression microarray for each of the 16 chromosomes and aligned according to chromosomal order. Red dots represent genes from chromosome III and gray from all other chromosomes. The vertical line within each chromosome represents the centromere position. Whereas in all chromosomes the average ration is around zero (indication for the identical chromosome copy number), for chromosome III there is ~50% expression relative to the WT, an indication of one copy of chromosome III compared with two copies in the WT.

Table S1. Heat tolerance contribution of genes from chromosome III introduced separately into a diploid wild type

ORF	Heat tolerance contribution, %	Gene name
Genes that retain elevated expression after the elimination of chromosome III trisomy in *Refined 1–4*		
YCR065W	23.5	HCM1
YCR016W	19.9	Uncharacterized
YCR045C	19.3	RRT12
YCR102C	18.8	Uncharacterized
YCR071C	18.4	IMG2
YCL005W-A	16.0	VMA9
YCL059C	13.6	KRR1
YCL035C	10.6	GRX1
YCR007C	8.3	Uncharacterized
YCL001W	7.2	RER1
YCL063W	6.4	VAC17
YCL036W	3.7	GFD2
YCR003W	2.6	MRPL32
YCR087C-A	0.9	LUG1
YCR043C	0	Uncharacterized
YCL061C	−1.7	MRC1
YCL026C-B	−2.2	HBN1
Genes that returned to wild-type expression level after the elimination of chromosome III trisomy in *Refined 1–4*		
YCL056C	−1.2	PEX34
YCL055W	−4.1	KAR4
YCL048W	−1.6	SPS22
YCL047C	−3.1	POF1
YCL045C	0.2	EMC1
YCL033C	0.3	MXR2
YCL032W	−1.9	STE50
YCL029C	−4.5	BIK1
YCL016C	1.8	DCC1
YCL004W	−0.5	PGS1
YCL001W-A	−3.1	Uncharacterized
YCL001W-B	1.5	Uncharacterized
YCR002C	−2.4	CDC10
YCR010C	−6.3	ADY2
YCR012W	−5.5	PGK1
YCR027C	0.4	RHB1
YCR031C	−5.0	RPS14A
YCR035C	−4.4	RRP43
YCR046C	1.3	IMG1
YCR047C	−5.2	BUD23
YCR086W	−4.4	CSM1
YCR101C	−5.4	Uncharacterized

See *SI Text* for heat tolerance functional analysis of these genes. Heat tolerance was measured by survival after 90-min exposure to 45 °C (*Materials and Methods*). The contribution of each gene was calculated by subtracting the tolerance of the wild type with an empty plasmid and then dividing by the tolerance of *WTtrisomeIII* (to define wild-type contribution as 0% and trisomeIII as 100%).

Table S2. List of mutations in high-pH strains

Gene	Mutation	Function
Mutation in EvoHigh-pH*		
GTT2/ MMP1	Converging 3′UTRs	GST/S-methylmethionine permease
YFR057w	Base substitution at promoter	Unknown
ECM21	Nonsense at codon 193	Ubiquitin-ligase adaptor
NMD4 /YLR363w-a	Base substitution at promoter	Nonsense-mediated mRNA decay/unknown
GPI17	S63 to L	GPI-anchor transamidase
YHR140w/ SPS100	Base substitution at promoter	Unknown/spore wall maturation
MAC1	C271 to W	Copper-sensing transcription factor
Mutation in *evoHigh-pH* spores (euploid and disomic)†		
GTT2/ MMP1	Converging 3′UTRs	GST/ S-methylmethionine permease
YFR057w	Base substitution at promoter	Unknown
GPI17	S63 to L	GPI-anchor transamidase
MAC1	C271 to W	Copper-sensing transcription factor

*Mutations found in the sequencing of *evoHigh-pH* obtained from Romano et al. (1).
†Subset of mutations commonly found in two spores of *evoHigh-pH*: one euploid and another with an extra copy of chromosome V.

1. Romano GH, et al. (2010) Different sets of QTLs influence fitness variation in yeast. *Mol Syst Biol* 6:346.